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Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,226,391**, on March 13, 1998, by **THE UNIVERSITTY OF BRITISH COLUMBIA**, assignee of Jiang-Hong Gong, Beatrice Dewald, Marco Baggiolini and Ian Clark-Lewis, for "N-Terminal Peptides of SDF-1 have Functional Activities Mediated by CXCR4".

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Abstract

Peptides corresponding to the N-terminal 9 residues of stromal cell derived factor-1 (SDF-1) have SDF-1 activity. Peptides, corresponding to residues 1-8, 1-9, and a disulfide linked dimer of 1-9, involving Cys-9, induced chemotaxis in T lymphocytes and CEM cells, and bound the SDF-1 receptor, CXC chemokine receptor 4 (CXCR4). The peptides had similar activities to SDF-1, but were less potent. Whereas native SDF-1 had half maximal chemoattractant activity at 5 nM, the 1-9 dimer and a 1-9 monomer analog were 100- and 3600-fold less potent, respectively. Receptor desensitization and competition binding experiments indicated that the SDF-1 peptides are specific for CXCR4. As Cys-9 is involved in a disulfide bridge with Cys 34 in native SDF-1, it is possible that the disulfide of the peptide dimer enhances its structural similarity to the native protein. Overall this study shows that the N-terminal region is sufficient to bind and activate CXCR4, which suggests the feasibility of designing small CXCR4 agonists or antagonists.

Introduction.

SDF-1 is a member of the chemokine family of structurally related proteins that function as cell chemoattractants (1). Although many chemokines have pro-inflammatory roles, SDF-1 appears to have a fundamental role in the trafficking export and homing of bone marrow cells (2, 3). It is produced constitutively, and particularly high levels are found in bone-marrow stromal cells (4, 5). A basic physiological role is implied by the high level of conservation of the SDF-1 sequence between species (4). In vitro SDF-1 stimulates chemotaxis of a wide range of cells including monocytes and bone marrow derived progenitor cells (2, 5). Particularly notable is its ability to stimulate a high percentage of resting and activated T lymphocytes (5, 6). It is the only known ligand for CXCR4, a 7 transmembrane receptor that has been variously described as LESTR (7), HUMSTR (8) and Fusin (9). CXCR4 is widely expressed on cells of hemopoietic origin, and is a major co-receptor for HIV-1 (9). Consistent with this dual role of CXCR4, SDF-1 blocks HIV-1 entry into CD4$^+$ cells (10, 11).

The SDF-1 sequence indicates that it belongs to the CXC family of chemokines, but has only about 22% identity with other chemokines (5). Despite the divergent primary structure, the recently described 3-dimensional structure indicates that it has the same overall fold as other chemokines (12). Furthermore, structure-activity analysis of SDF-1 (12) indicates the importance of N-terminal residues 8 for binding, and residues 1 and 2 were important for receptor activation. Residues 12-17 located in the loop region play a role in binding but not activation. In the SDF-1 structure, the region N-terminal to the CXC motif is highly disordered, but the loop region immediately following the CXC motif is well defined at least in its backbone atoms. These two regions have been identified as being important in other CC and CXC chemokines (12-15). As with other chemokines, N-terminal modification of SDF-1 led to dissociation of binding and activity (12). Thus despite the difference in primary structure, from both a structural and a functional perspective, the general mechanism of receptor binding is similar for SDF-1 and other chemokines.

The key role of the N-terminal region of the SDF-1 protein in receptor binding and activation suggests that the N-terminal region alone could be sufficient for binding or activity. Here we show that peptides corresponding to the N-terminal region bind CXCR4 and have SDF-1 activity. A dimer of SDF-1(1-9) was the most potent of the 4 peptides tested.

Experimental Procedures

Peptide Synthesis. The peptides were prepared as described previously (13). The peptides were purified by HPLC and analyzed by mass spectrometry. tBoc-α-aminobutyric acid was used to prepare the analog SDF-1(1-9)[Aba 9] which had CH_2-CH_3 in place of CH_2SH. The 1-9 peptide was dimerized via a disulfide bridge formed by gentle oxidation of the cysteines using 10% DMSO in water. Dimer formation was verified, following HPLC purification, by mass spectrometry.

Cell preparation and culture. Human peripheral blood mononuclear cells were isolated from donor blood buffy coats by centrifugation on Ficoll Paque. The cells were treated with phytohemagglutinin (1.0 $\mu g.ml^{-1}$) and expanded in the presence of IL-2 (100 $U.ml^{-1}$) for 7 to 17 days as described (16). These cells are referred to as "T lymphocytes". CEM cells, a human lymphoblastoid $CD4^+$ T cell line, was cultured in RPMI medium containing 10% FCS.

$[Ca^{2+}]_i$ changes. T lymphocytes and CEM cells loaded with Fura-2 were stimulated with the indicated agonist, and the $[Ca^{2+}]_i$-related fluorescence changes were recorded from 0-60 s (17). Receptor desensitization was tested by monitoring changes during sequential additions at 60 sec intervals

Chemotaxis. Migration of T lymphocytes was assessed in 48 well chambers (NeuroProbe, Cabin John, MD) using collagen-coated polyvinylpyrrolidone-free polycarbonate membranes with 3 μm pores (16). Migrated cells were counted in five randomly selected fields at 1000x magnification after migration of 1 h. Disposable Transwell chambers with a diameter of 6.5 mm and membrane pore size of 3 μm, were used to assay chemotaxis of CEM cells (Costar, Cambridge MA). The agonist, in Hepes-buffered RPMI 1640 supplemented with 10 $mg.ml^{-1}$ BSA (0.6 ml), was added to the lower well, and 0.1 ml of CEM cells ($1 \times 10^7.ml^{-1}$) in the same medium was added to the upper wells. After 3 h, cells that migrated to the lower wells were counted. Chemotactic migration was determined by subtraction of cells migrated in medium alone. All assays were performed in duplicate.

CXCR4 Receptor Binding. Competition for binding of ^{125}I-labeled SDF-1 to CEM cells was carried out as described (12). MCP-1 and RANTES binding was measured on THP-1 cells as documented elsewhere (14).

Results

N-terminal SDF-1 Peptides. The N-terminal residues of SDF-1 are critically involved in receptor recognition and activation (12). We have, therefore, examined chemically synthesized N-terminal peptides of SDF-1 for functional activity. These peptides corresponded to the first 8 or 9 residues of SDF-1. In addition to the 1-8 and 1-9 peptides, a dimer of 1-9, and analog 1-9[Aba-9] in which CH_2-CH_3 replaced the side-chain of cysteine, were prepared and tested for functional activity and receptor binding. The masses of the peptides and native SDF-1 were consistent with the sequences shown in Fig. 1.

SDF-1 N-terminal peptides are chemotactic. Both the 1-8 and 1-9 peptides induced dose-dependent chemotaxis of CEM cells (Fig. 2a). The concentrations required for 50% of the maximal response (EC50) are summarized in Table 1. The EC50 was 3.8×10^{-5} M for 1-8; 5.2×10^{-6} M for 1-9; and 5×10^{-9} M for native SDF-1. Thus the 1-9 peptide is about 1,000-fold less potent than native SDF-1. The 1-9 peptide was 7-fold more potent than the 1-8 peptide. The N-terminal peptides were also tested on T lymphocytes (Fig. 2b) and the results were similar to those obtained with CEM cells, except that T lymphocytes were somewhat less responsive to the N-terminal peptides. The results clearly show that the 1-9 and 1-8 peptides have SDF-1 like activity, but have relatively low potency. The chemoattractant activity of 1-9 was fully inhibited by the SDF-1 antagonist, SDF-1(1-67)[P2G] (12), but not by an IL-8 antagonist which blocks CXCR1 (18) as shown in Fig. 3. These findings further suggest that the 1-9 peptide is similar in its functional properties to native SDF-1.

In order to explore the possibility that the low potency of the N-terminal peptides is due to the lack of a second binding site, we tested whether activity could be enhanced by co-addition of a folded fragment corresponding to SDF-1(9-67), which lacks residues 1-8. Thus the entire SDF-1 structure was available to the receptor, but as two separate molecules. SDF-1(9-67) alone did not bind CXCR4 at the concentrations used (12). Chemotaxis, mobilization of cytosolic free calcium and receptor binding of 1-8

or 1-9 were not affected by the addition of SDF-1(9-67) (not shown). Thus no synergy could be demonstrated.

Activity of SDF-1(1-9) dimer. We tested two possible mechanisms that could account for the 7-fold difference between the 1-9 and 1-8 peptides: first whether the increase in length was responsible and second whether 1-9 forms a dimer via Cys-9 that is more active than 1-9 monomer. To determine if the extra residue in 1-9, was responsible for its higher activity we prepared an analog of 1-9 with Cys-9 replaced by Aba, a non-thiol-containing amino acid. The resulting peptide, 1-9[Aba-9] had similar activity to the 1-8 peptide, indicating that the extra residue alone was not the basis for the higher potency of 1-9 (Fig. 2a). As this 1-9[Aba] peptide could not dimerize via a disulfide bridge, we refer to it as the 1-9 monomer analog. Although the 1-9 used in these experiments was isolated as the monomer form, it has the potential to spontaneously form a disulfide linked dimer after isolation. To examine the possibility that the 1-9 formed a dimer with higher activity, we oxidized 1-9 to its disulfide bridged dimer form and isolated the dimer. The purified 1-9 dimer had higher activity than either 1-9 (10-fold); 1-8 (75-fold), or the 1-9[Aba-9] monomer analog (36-fold). The activity of 1-9 dimer was significant, but it was still 100-fold less potent than native SDF-1 (Table 1). Thus, the hypothesis that the 1-9 partially dimerizes accounting for its higher potency is correct.

Receptor binding of the SDF-1 peptides. CEM cells were used to determine the binding of the SDF-1 peptides to CXCR4 (12). The competition for binding of ^{125}I-labeled native SDF-1 by unlabelled native SDF-1 and the N-terminal peptides is shown in Fig. 4. The K_d values are summarized in Table 1. The competition by both the 1-8 and 1-9[Aba-9] peptides was incomplete, so a reliable K_d could not be determined. The 1-9 dimer peptide had 75-fold lower affinity than native SDF-1. In comparison the 1-9 peptide was 1500-fold lower affinity than native SDF-1. The binding data approximately corresponded with the chemotaxis results. To determine whether the N-terminal peptides bind to other chemokine receptors, competition for MCP-1 or RANTES binding to THP-1 cells was measured. THP-1 cells express CXCR4 as well as a number of CC chemokine receptors, including receptors for MCP-1 and

RANTES. Like native SDF-1, the peptides did not compete for the binding of either MCP-1 or RANTES (not shown).

SDF-1 peptides are specific for CXCR4. In agreement with the chemotaxis data, native SDF-1 and the N-terminal peptides induced a rapid and transient rise in cytoplasmic calcium concentration, $[Ca^{2+}]_i$, in T lymphocytes (Fig. 5a) as well as CEM cells (not shown). The rate and magnitude increased with the concentration. Whereas a response to SDF-1 was observed at 1×10^{-9} M, the peptides induced $[Ca^{2+}]_i$ changes in the micromolar range. Receptor usage by the SDF-1 peptides was assessed by monitoring $[Ca^{2+}]_i$ changes after sequential stimulation. As shown in Fig. 5a, treatment of T lymphocytes with SDF-1 completely abolished the responsiveness to the 1-9 peptide, and conversely, the 1-9 peptide also markedly attenuated the response to native SDF-1. The 1-9 dimer (50 μM) completely desensitized the response to subsequent native SDF-1 (not shown). No effect on the response to the 1-9 peptide was observed when T lymphocytes were pre-stimulated with MCP-1, RANTES, MIP-1β, IP10, or Mig (Fig. 5b). The selectivity of these chemokines (1) implies that SDF-1 peptides desensitize CXCR4 but not other chemokine receptors including CXCR3, CCR1, CCR2 and CCR5. No response to eotaxin, I-309 or TARC (Fig. 5b) was obtained with these cells under the conditions used, and as expected, they did not desensitize 1-9. Taken together these data show that the 1-9 peptide binds and activates CXCR4, and in addition indicate that it is specific for CXCR4.

Discussion.

We have shown that N-terminal peptides of SDF-1 activate CXCR4. Peptides corresponding to 1-8, 1-9 and the dimer of 1-9 bind CXCR4 and stimulate SDF-1 functions. Although residues in the N-terminal region of other chemokines are generally critical for receptor-activation, N-terminal peptides do not bind and stimulate chemokine function. Thus SDF-1 is an exception in its minimal requirement for receptor activation.

Experiments in which hybrid chemokines were prepared with the N-terminal 8 residues of SDF-1 "cut and pasted" into the corresponding position of the CXC chemokines, GROα or IP10, showed that the SDF-1 N-terminal motif was sufficient to confer SDF-1 activity on to these chemokines (12). Truncation and substitution experiments supported the conclusion that the N-terminal domain of SDF-1 contains the major determinants of CXCR4 binding and activation. Modification of only the N-terminal 2 residues of SDF-1 resulted in CXCR4 antagonists indicating that the receptor activation site is in the N-terminal two residues (12). These experiments demonstrated the critical role of the N-terminal region in SDF-1 function, and suggested the possibility that the N-terminal peptides alone could be functional.

The disulfide-linked dimer of SDF-1(1-9) peptide was considerably more potent than the 1-9 peptide monomer. A cysteine modified form, 1-9[Aba-9], that did not form a dimer was also active, but 3-fold less potent than the 1-9 peptide. This indicates that the cysteine is important for the higher potency of the 1-9, and it is likely that this is due to spontaneous partial dimer formation in solution. In native SDF-1, Cys-9 normally participates in a disulfide bridge between residues 9 and 34. Thus, the disulfide bridge of the 1-9 dimer could directly enhance binding to the receptor. A second alternative is that dimerization could change the conformation of the 1-9 binding motif resulting in an enhanced binding affinity. A third possibility is that one half of the dimer binds the activation site, but the other half could bind to other receptor sites, perhaps due to similarities in the positive charge of the N-terminal motif and other binding sites on the SDF-1 protein. Further experiments will be required to determine the mechanisms involved. The fact that native SDF-1 binds as a monomer, and not as a dimer (12), indicates that only one 1-9 motif

normally binds to the receptor site, however, our experiments have not formally ruled out the possibility that the dimer binds at two separate sites on one receptor, or cross-links two receptors. This is unlikely from the forgoing discussion of the mechanism of action of the 1-9.

The mechanism of CXCR4 activation by the SDF-1 peptides is suggested by the recently solved 3D structure, and structure-activity relationships of the native protein (12). The ^{1}H-NMR structure of SDF-1 shows that the N-terminal region is entirely solvent accessible and has no detectable protein-protein interactions and therefore is expected to be highly flexible. It is reasonable to propose that the N-terminal region establishes specific interactions, and adopts a well defined conformation when SDF-1 binds to CXCR4. When the N-terminal region is represented as a peptide in solution then it would be expected to be solvent exposed and flexible, and also adopt a receptor bound conformation.

There are two possible reasons for the difference between the N-terminal peptides of SDF-1 and those of other chemokines. First, a substantial body of work had lead to a model in which there are two chemokine receptor interaction sites, the initial interaction occurs with a site in the loop region that follows the CXC or CC (12-15, 18-20). This interaction facilitates the subsequent binding of the N-terminal region to a buried site in the receptor (12, 15). Therefore interaction of the loop region with the receptor could be required for activity of these other N-terminal peptides (15). SDF-1 also exhibits binding in the loop region. However, the activity of the N-terminal peptides demonstrate that in the case of SDF-1, the loop is not an absolute requirement. The second possible reason for the lack of binding of the N-terminal peptides of chemokines other than SDF-1 is that in the absence of the native protein context, they did not adopt a receptor bound conformation. Chemokines have two disulfide bridges that anchor the N-terminal region to the remainder of the protein structure. Both disulfide bridges are important for function, and we have proposed that they provide an essential scaffold that allows the N-terminal region and the loop region to adopt the optimal bound conformation.

The affinity and potency of the N-terminal SDF-1 peptides are lower than those of the native protein. Thus the peptides can bind CXCR4, but not as efficiently as native SDF-1. Similar arguments to those

above could account for the difference in potency. The low affinity could be due to the lack of the loop binding site on the N-terminal peptide or to suboptimal conformation of the peptide. So far no attempts have been made to optimize the function of SDF-1 peptides by changing their covalent structure.

Stable low molecular weight non-peptide ligands are preferred for therapeutic applications. SDF-1 is the co-receptor for HIV and is involved in hemopoietic cell homing. Several non-chemokine molecules have been found to inhibit HIV and it was shown that they target CXCR4 (21-23). However none of these have SDF-1 activity. Antagonists of chemokines are likely to be the most useful variants for therapeutic usage, and we have demonstrated that modification to N-terminal sites of several chemokines, including SDF-1, results in antagonists. Many 7-transmembrane receptors have small molecule natural ligands and have been successfully targeted by analogs to generate pharmaceutical compounds. However protein ligands such as chemokines, which have larger binding surfaces and depend on cooperative interactions present a more complex chemical problem. The results with SDF-1 peptides indicate that it would be feasible to target the N-terminal region alone. These peptides could be leads for the generation of low molecular weight high affinity CXCR4 agonists or antagonist.

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References

1. Baggiolini, M., Dewald, B., and Moser, B. (1997). *Ann. Rev. Immunol.* 15, 675-705

2. Aiuti, A., Webb, I. J., Bleul, C., Springer, T., and Guierrez-Ramos, J. C. (1996) *J. Exp. Med.* 185, 111-120

3. Nagasawa, T., Hirota, S., Tachibana, K., Takakura, N., Nishikawa, S.-I., Kitamura, Y., Yoshida, N., Kikutani, H., and Kishimoto, T. (1996) *Nature* 382, 635-638

4. Shirozu, M., Nakano, T., Inazawa, J., Tashiro, K., Tada, H., Shinohara, T., and Honjo, T. (1995) *Genomics* 28, 495-500

5. Bleul, C. C., Fuhlbrigge, R. C., Casasnovas, J. M., Aiuti, A., and Springer, T. A. (1996) *J. Exp. Med.* 184, 1101-1109

6. Campbell, J. J., Hendrick, J., Zlotnik, A., Siani, M. A., Thompson, D. A., and Butcher, E. C. (1998) *Science* 279 381-383

7. Loetscher, M., Geiser, T., O'Reilly, T., Zwahlen, R., Baggiolini, M., and Moser, B. (1994) *J. Biol. Chem.* 269, 232-237

8. Federsppiel, B., Duncan, A. M. V., Delaney, A., Schappert, K., Clark-Lewis, I., and Jirik, F. R. (1993) *Genomics* 16, 707-712

9. Feng, Y., Broeder, C. C., Kennedy, P. E., and Berger, E. A. (1996) HIV-1 entry cofactor: Functional cDNA cloning of a seven-transmembrane G protein-coupled receptor. *Science* **272**, 872-877

10. Oberlin, E., Amara, A., Bachelerie, F., Bessia, C., Virelizier, J.-L., Arenzana-Seisdedos, F., Schwartz, O., Heard, J.-M., Clark-Lewis, I., Legler, D. F., Loetscher, M., Baggiolini, M., and Moser, B. (1996) *Nature* **382**, 833-835

11. Bleul, C. C., Farzan, M., Choe, H., Parolin, C., Clark-Lewis, I., Sodroksi, J., and Springer, T.A. (1996) *Nature* **382**, 829-833

12. Crump, M., Gong J.-H., Loetscher, P., Rajarathnam, K., Amara, A., Arenzana-Seisdedos, F., Virelizier, J.-L., Baggiolini, M., Sykes, B.D., and Clark-Lewis, I. (1997) *EMBO J.* **16**, 6996-7007

13. Clark-Lewis, I., Dewald, B., Loetscher, M., Moser, B., and Baggiolini, M. (1994) *J. Biol. Chem.* **269**, 16075-16081

14. Gong, J.-H., Uguccioni, M., Dewald, B., Baggiolini, M., and Clark-Lewis, I. (1996) *J. Biol. Chem.* **271**, 10521-10527

15. Clark-Lewis, I., Kim, K.-S., Rajarathnam, K., Gong, J.-H., Dewald, B., Moser, B., Baggiolini, M., and Sykes, B.D. (1995) *J. Leukocyte Biol.* **57**, 703-711

16. Loetscher, P., Seitz, M., Clark-Lewis, I., Baggiolini, M., and Moser, B. (1994) *FASEB J.* **8**, 1055-1060

17. von Tscharner, V., Prod'hom, B., Baggiolini, M. and Reuter, H. (1986) *Nature* **324**, 369-372

18. Jones, S. A. , Dewald, B., Clark-Lewis, I., and Baggiolini, M. (1997) *J. Biol. Chem.* **272**, 16166-16169

19. Monteclaro, F. S., and Charo, I. F. (1996) *J. Biol. Chem.* **271**, 19084-19092

20. Lowman, H. B., Slagle, P. H., DeForge, L. E., Wirth, C. M., Gillee-Castro, B. L., Bourell, J. H., and Fairbrother, W. J. (1996) *J. Biol. Chem.* **271**, 14344-14352

21. Doranz, B. J., Grovit-Ferbas, K., Sharron, M. P., Mao, S.-H., Goetz, M. B., Daar, E. S., Doms, R. W., and O'Brien, W. A. (1997) *J. Exp. Med.* **186**, 1395-1400

22. Murakami, T., Nakajima, T., Koyanagi, Y., Tachibana, K., Fujii, N., Tamamura, H., Yoshida, N., Waki, M., Matsuoto, A., Yoshie, O., Kishimoto, T., Yamamoto, N., and Nagasawa, T. (1997) *J. Exp. Med.* **186**, 1389-1393

23. Schols, D., Struyf, S., Van Damme, J., Estè, J. A., Henson, G., and Clercq, E. D. (1997) *J. Exp. Med.* **186**, 1383-1388

Footnotes

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‡ Contributed equally to this work.

1 Abbreviations used:

SDF-1, stromal cell derived factor-1; Aba, α-amino-(n)-butyric acid; CXCR, CXC chemokine receptor; CCR, CC chemokine receptor; $[Ca^{2+}]_i$, intracellular concentration of calcium ions; GROα, growth related protein-α; IL-8 interleukin-8; IP10, γ-interferon inducible protein-10; Mig, monokine induced by interferon-γ; MIP-1β, macrophage inflammatory protein-1β; RANTES, regulated on activation normal T cell expressed; TARC, thymus and activation-regulated chemokine.

Table 1. Summary of the relative potencies of SDF-1 peptides

	Binding		Chemotactic activity	
Peptides	**K_ds (nM)[a]**	**Fold increase[b]**	**EC50 (nM)[c]**	**Fold increase[d]**
SDF-1	9 ± 3	1	5 ± 1	1
SDF, 1-8	e		37,500 ± 10,600	7,500
SDF, 1-9	13,900 ± 5,500	1,500	5,200 ± 3,800	1,040
SDF, 1-9[Aba-9]	e		17,800	3,600
SDF, 1-9 Dimer	670 ± 110	75	500	100

[a] K_ds were calculated from CEM cell binding curves derived in 2-6 experiments, with results similar to those in Fig. 4, using Scatchard methods.

[b] The fold increase in K_d was calculated relative to native SDF-1.

[c] The chemotaxis EC50 was calculated from the CEM cell data in Fig. 2*a*. Results are presented as the mean ± SD from 2 experiments.

[d] Fold increase in chemotaxis EC50 was calculated relative to native SDF-1.

[e] K_d not determined (see text).

Figure legends

Figure 1. Sequences of native SDF-1 and the N-terminal SDF-1 peptides.

Figure 2. Chemoattractant activity of SDF-1 peptides. Concentration dependent migration of CEM cells*(a)*; and T-lymphocytes *(b)*. in response to the SDF-1 peptides: 1-8 (❑); 1-9 (Δ); 1-9 dimer (▲); and 1-9[Aba] (⊞); and in response to native SDF-1 (●). Data are shown is the mean ± SD of migrated cells. Similar results were obtained in two additional experiments

Figure 3. Chemotaxis inhibition by chemokine antagonists. CEM cell migration induced by SDF-1(1-9) peptide (10 μM) in the presence of the indicated concentrations of the SDF-1 antagonist, SDF-1(1-67)[P2G] (❒); or the IL-8 antagonist, IL-8(6-72) (○). Migration is expressed as percent of the response obtained in the absence of antagonist (control, ■). Data are the means ± SD of duplicate determinations from 2 separate experiments.

Figure 4 Receptor binding of SDF-1 peptides. Competition for specific binding of ^{125}I-SDF-1 (4 nM) to CEM cells by 1-8 (❑); 1-9 (Δ); 1-9 dimer (▲); 1-9[Aba-9] (⊞); native SDF-1 (●). The percentage specific cpm bound in the absence of competitor (■), is shown. Representative results from 2 to 6 experiments.

Figure 5. Receptor selectivity of the SDF-1 peptides. T lymphocytes that were loaded with Fura-2 were sequentially stimulated with chemokines and SDF-1(1-9) and the resulting $[Ca^{2+}]_i$ dependent fluorescence changes were recorded. (a) Cross-desensitization of SDF-1 and the 1-9 peptide. (b) Lack of desensitization of SDF-1(1-9) by the indicated CXC or CC chemokines. The chemokines were added at 100 nM, except for SDF-1 which was added at 1 nM, followed by addition of the 1-9 peptide (30 μM) after 60 s. The results shown are representative of 2-3 independent experiments.

FIGURE 1

```
                    1          11         21         31
                    |          |          |          |
SDF-1               KPVSLSYRCP CRFFESHVAR ANVKHLKILN TPNCALQIVA

                              41         51         61
                              |          |          |
                              RLKNNNRQVCI DPKLKWIQEY LEKALN
```

SDF-1 (1-8) KPVSLSYR

SDF-1 (1-9) KPVSLSYRC

SDF-1 (1-9)[Aba] KPVSLSYRAba

SDF-1 (1-9) dimer

```
KPVSLSYRC
        |
KPVSLSYRC
```

Fig. 1

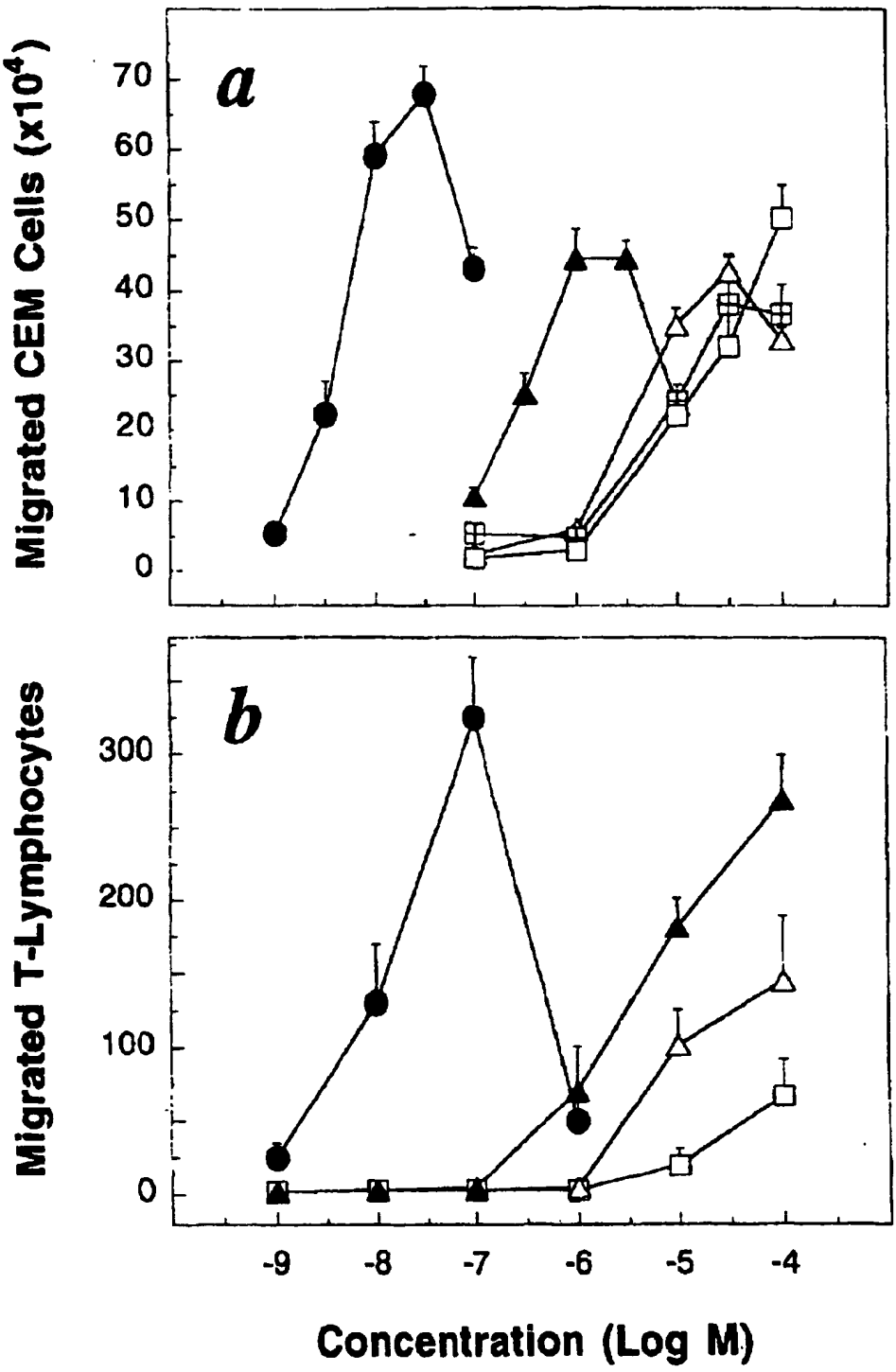
a
b
Migrated CEM Cells (x10⁴)
Migrated T-Lymphocytes
70
60
50
40
30
20
10
0
300
200
100
0
-9
-8
-7
-6
-5
-4
Concentration (Log M)

Fig. 2

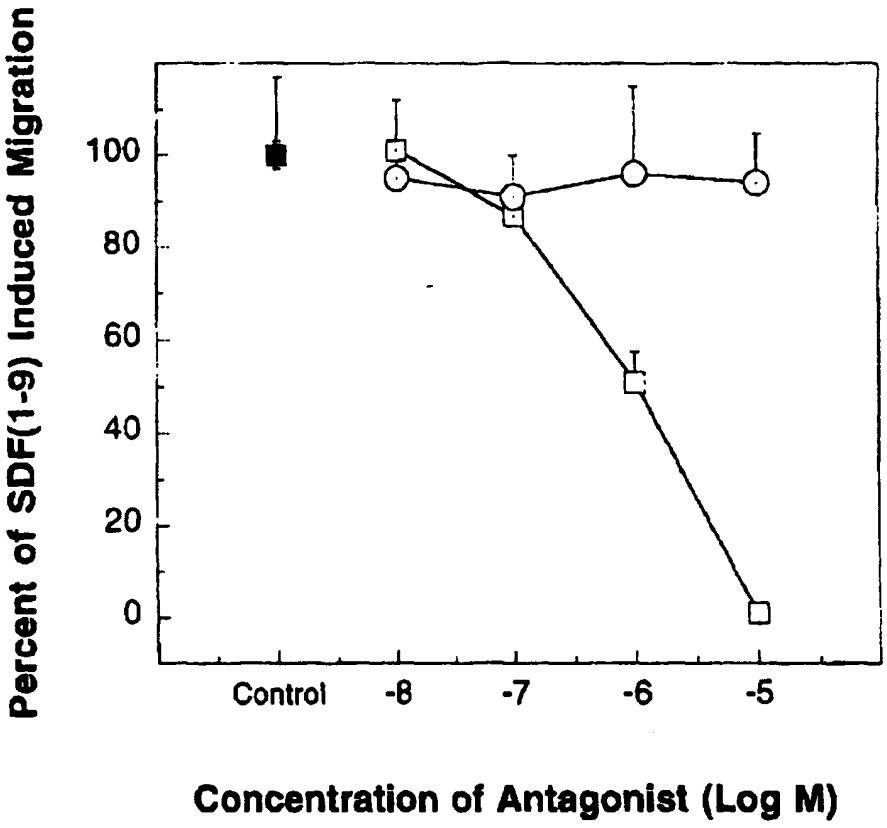
Percent of SDF(1-9) Induced Migration
100
80
60
40
20
0
Control
-8
-7
-6
-5
Concentration of Antagonist (Log M)

Fig. 3

Fig. 4

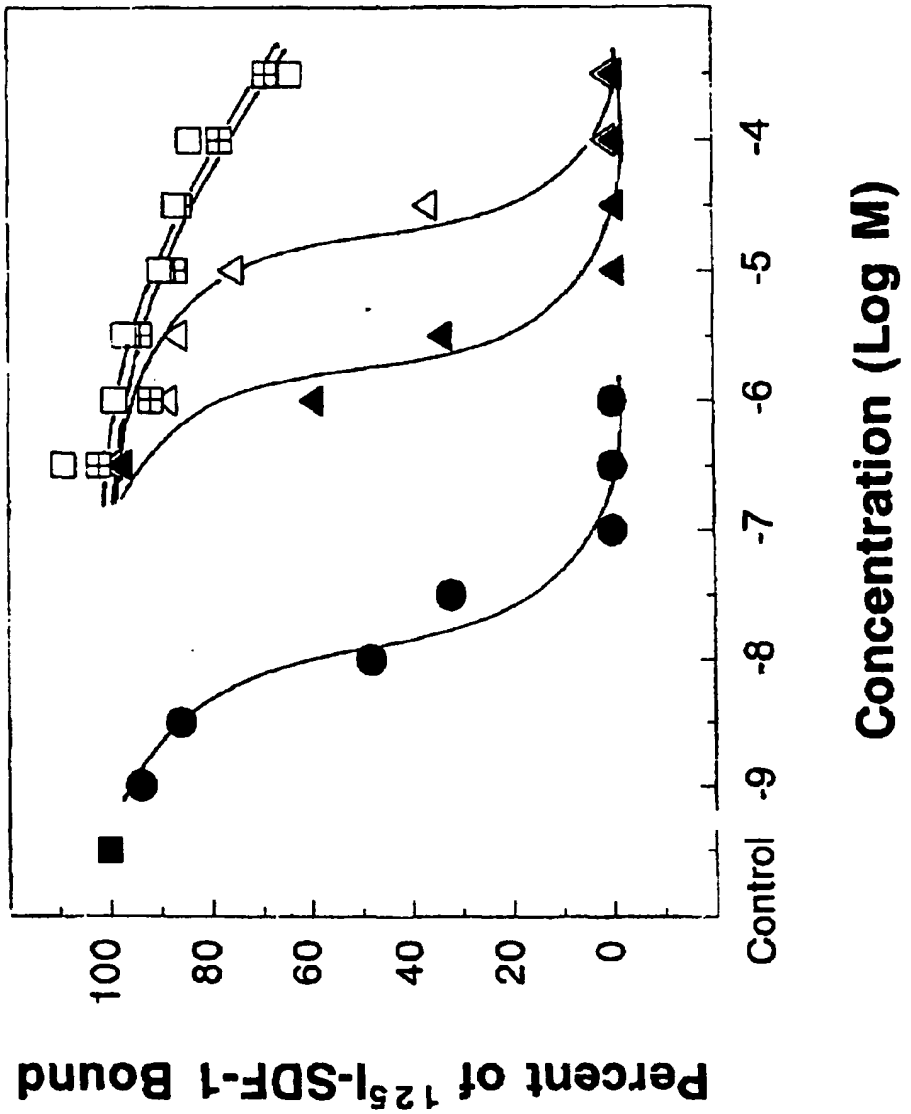
Percent of ^{125}I-SDF-1 Bound
100
80
60
40
20
0
Control
-9
-8
-7
-6
-5
-4
Concentration (Log M)

Fig. 4

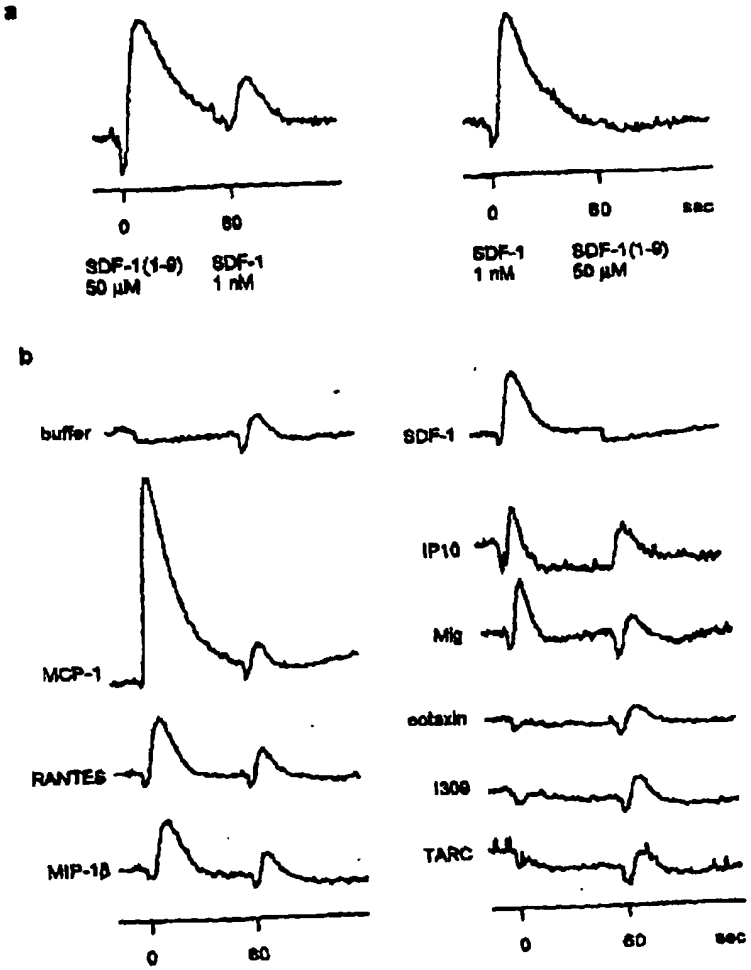
a
0
60
SDF-1(1-9)
50 µM
SDF-1
1 nM
0
60
sec
SDF-1
1 nM
SDF-1(1-9)
50 µM
b
buffer
SDF-1
IP10
Mig
MCP-1
eotaxin
I309
RANTES
TARC
MIP-1β
0
60
0
60
sec

Fig. 5